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Assessment of Antioxidant Activities of Kenaf Leaves (*Hibiscus cannabinus*) Extracts

S. Ibrahim, S. A. I. A. Syed Abdul Kadir, N. Ismail and **N. H. Ismail**

Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

ABSTRACT The antioxidant properties of petroleum ether extract [PE], dichloromethane fraction [DCM frac] and methanol crude extract [MeOH] of simple leaves of kenaf (*Hibiscus cannabinus*), Malvaceace family have been measured by Ferric thiocyanate (FTC) and Thiobarbituric acid (TBA) methods as described by Kikuzaki and Nakatani [1]. Other methods used for antioxidant assay are 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and reducing power [2]. FTC and TBA method for all crude extracts at 0.02% concentration showed low absorbance values, which indicates high antioxidant activities, as compared to vitamin E and quercetin. DPPH activity of [PE] extracts and [MeOH] at both 500 μg/ml, exhibited moderate activity at 50.15% and 66.93% inhibitions, respectively. However, reducing power showed low activity for all crude extracts.

(Antioxidant, *Hibiscus cannabinus*, Kenaf)

INTRODUCTION

Kenaf (*Hibiscus cannabinus*) is a member of the Malvaceae family. This herbaceous plant has been cultivated in Africa, Asia and Mediterranean areas. It was used in traditional medicine such as in venomous mushroom. It is also widely used in pulp and paper industries since its core and bark are source of fibers. Its also has good features to be used in several applications such as antidotes to chemical poisoning [3, 4]. In previous phytochemical investigations on kenaf, Seca *et al.* [5] reported the aliphatic composition of the bark and core. They also isolated new lignans from the core. Allelophatic properties of kenaf provide initial evidence for kenaf to be used as mulch in farming to reduced weed populations. Extracts from kenaf leaves also decreased germination of redroot pigweed, tomato and cucumber [5]. Kobaisy *et al.* [6] investigated that the essential oil and the major components from leaves of kenaf showed moderate phytotoxic activities and no antifungal activities. Younes *et al.* [7] investigated 10 volatile constituents from the leaves of *Hibiscuss cannabinus* collected mainly in Egypt. The seeds of kenaf are made up of glycerides of stearic, palmatic, oleic and linoleic acids. They are suitable to be used in the manufacture of linoleum paints and varnishes [8].

MATERIALS AND METHODS

General

Shidmazu UV-VIS Spectrophotometer model UV-1601 PC.

Chemicals

Vitamin E, Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), Quercetin and DPPH (Sigma-aldrich, CO. St. Louis, MO USA), Linoleic acid (Fluka Chemie Gimbh, Switzerland), Vitamin C (Dr Ehrenstorfer GmbH) Absolute Ethanol 99.7% (Hayman Limited, Essex UK)

Plant Material and Preparation of Extracts

Fresh kenaf leaves, which were harvested at maturity stage, were obtained from MARDI, Serdang Selangor. The simple leaves of kenaf were air-dried at room temperature. Then they were finely ground (447g) and steeped twice overnight in petroleum ether. The combined petroleum ether was evaporated at a reduce pressure to produced crude extract of petroleum ether with 21.57g (4.82% w/w) [PE]. The residue from petroleum ether extract was further steeped overnight in methanol for three times. The combined alcoholic extract was concentrated and gives 35.7g (8% w/w) [MeOH]. The MeOH extract was subjected to liquid-liquid partitioning

with 100 ml of dichloromethane (DCM) for three times. The DCM extract was concentrated under reduced pressure to give 5.65 g of crude extract.

Antioxidant assay

1. Sample preparation

For FTC and TBA assays the sample was prepared as followed [1]. In a screw-cap vial (∅ 38 mm x 75 mm) containing a mixture of 4 mg of sample, 4 ml of 99.5% ethanol, 4.1 ml of 2.5% of linoleic acid in 99.5% ethanol, 8 ml of 0.05M (pH 7) of phosphate buffer and 3.9 ml of water were mixed thoroughly. The mixed solution was incubated at 40°C for 8 to 10 days until one day after the absorbance of the control vial reached a maximum.

2. Ferric thiocyanate (FTC) method

To 0.1 ml of the sample mixture was added 9.1 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the amount of peroxide was determined by measuring the absorbance of the red color developed at 500 nm. The tests were carried out in triplicate. The measurements were taken every 24 hr for 8 to 10 days (until one day before the absorbance of the control vial reached a maximum).

3. Thiobarbituric acid (TBA) method

To 2 ml of sample solution was added with 1 ml of 20% aqueous trichloroacetic acid TCA and 2 ml of aqueous thiobarbiturc acid TBA solution. The mixture was heated in boiling water, cooled and centrifuged at 3000 rpm for 30 min before measuring the absorbance at 532 nm. Each extract were carried out in triplicate. Antioxidant activity was based on the absorbance of the final day of FTC assay.

4. DDPH radical scavenging activity

This assay was followed according to the method described by Yildirim *et al.* [2]. 1 ml of 1 mM of DPPH solution in absolute ethanol was mixed with 3 ml of extract solution in absolute ethanol. A range of sample concentration was prepared by doing a serial dilution from an initial concentration of 500 μg/ml. The absorbance of mixture was measured spectrophotometrically at 517 nm after incubated in a dark place for 30 minutes. Triplicate measurement was made and the antioxidant activity indicated by calculating the percentage inhibition of DPPH radicals.

5. Reducing power

This test was conducted according to the method described by Yildirim *et al.* [2]. 1 ml of extracts solution from a serial dilution with ranging of concentration of sample (500 – 7.81 μg/ml) in absolute ethanol were mixed with 2.5 ml of phosphate buffer (0.2 m, pH 6.6) and 2.5 ml of 1% of potassium ferricyanide [$K_3Fe(CN)_6$] and the mixture was incubated at 50°C for 30 minutes. Then, 2.5 ml of 10% trichloroacetic acid (TCA) was added and the mixture was centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of supernatant was mixed with 2.5 ml distilled water and 0.5 ml of $FeCl_3$ (0.1%). The absorbance was read spectrophotometrically at 700 nm. High absorbance values indicate high reducing power.

RESULTS AND DISCUSSION

Antioxidant activities based on lipid peroxidation

In present study, the kenaf leaves were evaluated for their antioxidant properties by using two methods as mentioned above. In the thiocyanate method, the lipids oxidation was determined by measuring the amount of peroxides formed during incubation of sample. During the oxidation process, peroxide is gradually decomposed to lower molecular compounds. The high absorbance of peroxides in emulsion with high concentration showed low antioxidant activity. While the low absorbance give the high antioxidant activity. In this work, all crude extracts exhibited very strong activity. Figure 1 illustrated the absorbance versus incubation time showing the activities of all crude extracts compared to standards for 7 consecutive days. Figure 2 shows the absorbance values on the last day of the assay (6^{th} day), which was one day after the control reached its maximum. The absorbance values of all tested kenaf extracts remained below 0.2 indicating very strong antioxidative properties comparable to antioxidative effect of Vitamin E (absorbance 0.4) and quercetin (absorbance 0.17). All the crude extracts are very active in inhibiting peroxidation.

Another method to monitor oxidation of lipids is the thiobarbituric acid (TBA). Degradation of peroxides during incubation is measured

spectrophotometrically at 532 nm. By determining the amount of lower molecules formed during the decomposition of peroxide, the correlation between both FTC and TBA methods can be verified. The low absorbance values of all crude extracts show high antioxidant activity as shown in Figure 3. These results correlated well with results obtained by FTC method.

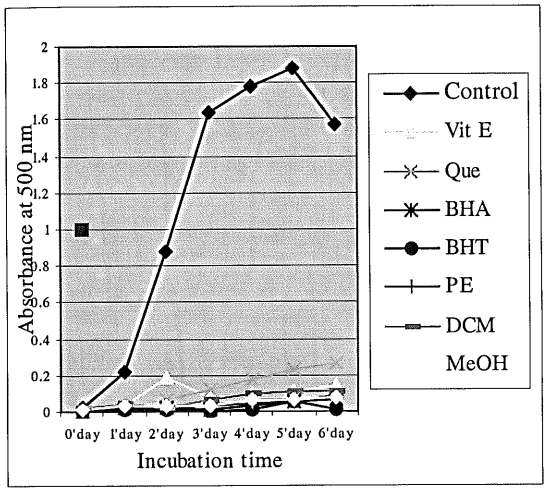

Figure 1. Graph of absorbance vs incubation time of antioxidant activity of Kenaf leaves extracts measured by FTC method

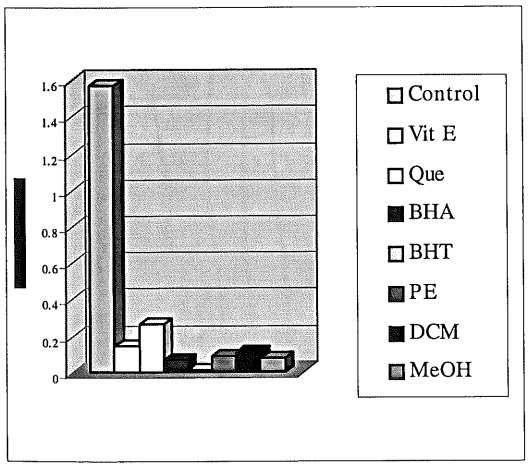

Figure 2. Absorbance values (on the 6th day) of different extracts of Kenaf leaves using FTC method

Table 1. Absorbance values of different extracts of Kenaf leaves using TBA method

	Absorbance at 532 nm (last day)
Control	1.2692
Vit E	0.0857
Quercetin	0.3585
BHA	0.1295
BHT	0.4223
PE	0.042
DCM	0.3167
MeOH	0.104

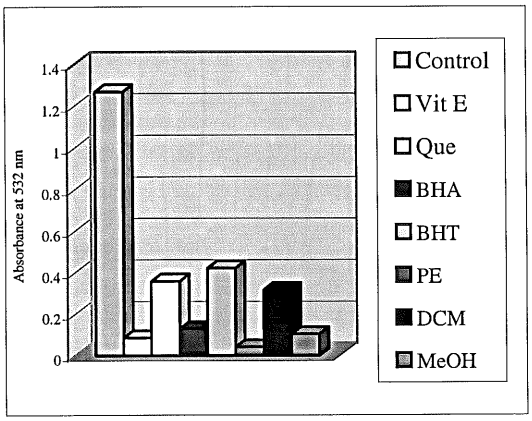

Figure 3. Absorbance values (on the 6th day) of different extracts of Kenaf leaves using TBA method

DPPH radical scavenging activity

DPPH radical scavenging activity was determined by measuring the disappearance of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometrically at 517 nm. The percentage inhibition can be expressed as a percentage reduction of the initial of DPPH absorption by test of extracts solution. The mechanism is well known. The potential of compounds/extracts to scavenger free radicals is due to the ability of the molecule to donate a hydrogen atom [9]. In the current work, the serial dilution of the concentrations (500, 250, 125, 62, 31, 15, 8 μg/ml) of petroleum ether extracts [PE], dichloromethane fraction [DCM] and methanol crude extract [MeOH] for simple leaves was perform to identify the radical scavenging activity. As shown in Figure 4, the DDPH scavenging activity of both petroleum ether crude extracts and methanol crude extract at the concentration of 500 μg/ml shows 50.15% 66.93% inhibition respectively. All crude extracts

showed a gradual decrease in activity by reducing the concentrations. This is showed that the percent inhibitions of DDPH radical of all crude extracts were concentration dependent as tabulated in Table 2. The standards were known synthetic antioxidants. There is no significant difference among the standards used, which shown high inhibition percentage even for the concentration as low as 62.5 µg/ml.

Table 2. Percent inhibition of DPPH scavenging activity at different concentration of Kenaf leaves extracts

	Absorbance at 517 nm			
	500 µg/ml	**250 µg/ml**	**125 µg/ml**	**62.5 µg/ml**
Vit E	89.17	88.15	89.94	89.21
Vit C	93.41	93.18	93.03	92.96
Que	90.51	89.89	89.82	89.49
BHA	89.87	89.89	90.69	89.49
PE	50.15	30.93	19.31	11.53
DCM	31.93	23.77	14.42	0
MeOH	66.93	38.76	28.25	15.52

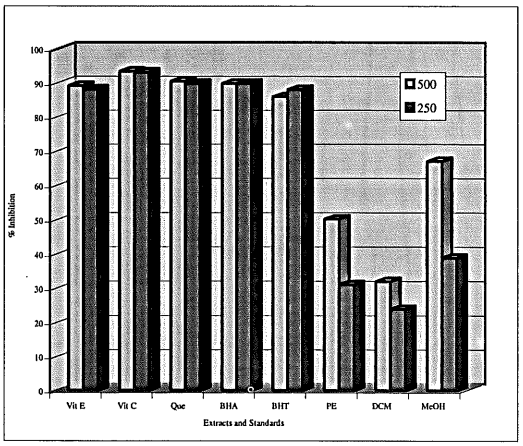

Figure 4. Percent DPPH scavenging activity of different extracts of Kenaf leaves at 500 and 250 µg/ml

Reducing power

All crude extracts exhibited low reducing activity. Figure 5 shows that the concentration of crude extracts was plotted against absorbance measured at 700 nm. It showed that the reducing power is concentration dependent. However, in our study we noticed that reducing power was in the following order PE > DCM > MeOH. Even the concentration 500 µg/ml, all crude extracts still showed low absorbance as plotted in Figure 6. Yildirim *et al.* [2], reported that the reducing power increased when the phenolic compounds in the sample increased. This is due to the fact that the reducing power of the compounds is related to its electron transfer ability. Therefore, we predict the amounts of phenolic compounds in the kenaf leaves are low.

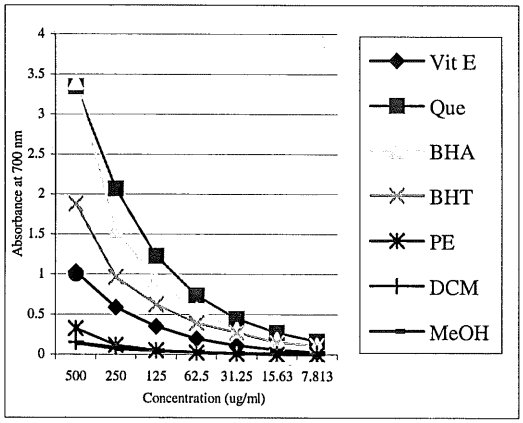

Figure 5. Reducing power of Kenaf leaves extracts

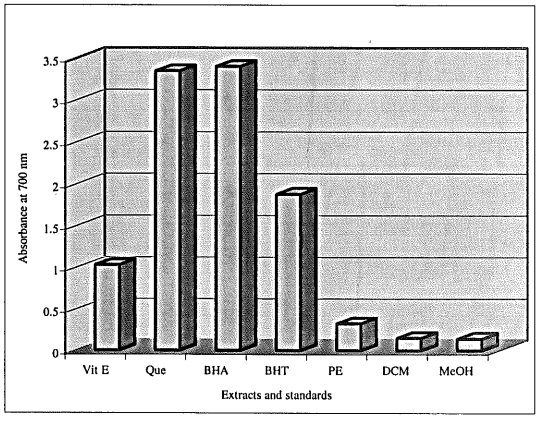

Figure 6. Absorbance values of different extracts of Kenaf leaves at 500 µg/ml

CONCLUSION

All crude extracts of the kenaf leaves showed good antioxidant activity when tested using Ferric thiocyanate (FTC) and Thiobarbituric acid (TBA) methods at concentration 0.02%. The [PE] and [MeOH] extracts showed moderate radical scavenging activity at concentration of 500 μg/ml.

REFERENCES

1. Kikuzaki, H. and Nakatani, N. (1993). *Journal of Food Science*. **58** (6): 1407-1410.
2. Yildirim, A., Mavi, A., and Kara, A. A. (2001). *Journal of Agricultural and Food Chemistry*. **49**: 4083-4089.
3. Seca, A. M. L., Silva. A. M. S., Silvestre, A. J. D., Cavaleiro, J. A. S., Domingues, F. M. J., Pascoal-Neto, C. (2001). *Phytochemistry*. **56**: 759-767.
4. Seca, A. M. L., Silva. A. M. S., Silvestre, A. J. D., Cavaleiro, J. A. S., Domingues, F. M. J., Pascoal-Neto, C. (2001). *Phytochemistry*. **58**: 1219-1223.
5. Seca, A. M. L., Silva. A. M. S., Silvestre, A. J. D., Cavaleiro, J. A. S., Domingues, F. M. J., Pascoal-Neto, C. (2000). *Phytochemical Analysis* **11**: 76.
6. Kobaisy, M., Tellez, M. R., Charles L. (2001). *J. Agric. Food Chem.* **49**: 3768-3771.
7. Younes, E. G. *et al.* (1976). *J. Chem* **19**: 633-641.
8. Burkill, I. H. (2002). *A Dictionary of the Economic Products of the Malay Peninsular*, p. 1182-1184.
9. Espin, J. C., Soler-Rivas, C., and Wichers, H. J. (2000). *Journal of Agricultural and Food Chemistry*. **48**: 648-656.